Ventilatory restraint of sympathetic activity during chemoreflex stress

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Steinback CD, Breskovic T, Frances M, Dujic Z, Shoemaker JK. Ventilatory restraint of sympathetic activity during chemoreflex stress. Am J Physiol Regul Integr Comp Physiol 299: R1407–R1414, 2010. First published September 8, 2010; doi:10.1152/ajpregu.00432.2010.—The within-breath modulation of muscle sympathetic nerve activity (MSNA) is well established, with greater activity occurring during expiration and less during inspiration. Whether ventilation per se affects the longer-term (i.e., minute-to-minute) regulation of MSNA has not been determined. We sought to define the specific role of ventilation in regulating sympathetic activation during chemoreflex activation, where both ventilation and MSNA are increased. Ten young healthy subjects performed both asphyxic rebreathing and repeated, rebreathing apneas to cause the same magnitude of chemoreflex stress in the presence or absence of ventilation. Both protocols caused increases in sympathetic burst frequency, burst amplitude, and burst incidence. However, burst frequency was increased more during repeated apneas (12 ± 6 to 25 ± 7 bursts/min) compared with rebreathing (12 ± 5 to 17 ± 7 bursts/min; P < 0.001) due to a greater burst incidence during apneas (36 ± 11 bursts/100 heart beats) vs. rebreathing (26 ± 8 bursts/100 heart beats, P < 0.001). The sympathetic gain to chemoreflex stress was also larger during repeated apneas (2.29 ± 1.29 au%/desaturation) compared with rebreathing (1.44 ± 0.53 au%/desaturation, P < 0.05). The augmented sympathetic response during apneas was associated with a larger pressor response and total peripheral resistance compared with rebreathing. These data demonstrate that ventilation per se restrains sympathetic activation during chemoreflex activation. Further, the augmented sympathetic response during apneas was associated with greater cardiovascular stress and may be relevant to the cardiovascular pathology associated with sleep-disordered breathing.

rebreathing; apnea; microneurography

THE PHYSIOLOGICAL RESPONSE to peripheral and/or central chemoreflex stimulation involves both corrective and compensatory mechanisms. The corrective mechanisms include an augmented ventilatory response designed to increase alveolar, and, in turn, arterial, PO2 and decrease alveolar and arterial PCO2. The compensatory mechanisms, on the other hand, include an increased sympathetic vasoconstrictor response to redistribute blood flow to critical organs and tissues. Using this model, we found that it was apparent that the role of compensatory sympathetic activation is dependent on the effectiveness of the ventilatory response in correcting blood gases. However, the potential impact of ventilation per se on sympathetic regulation in this scenario is less obvious.

The role of ventilation in modulating sympathetic nervous activity to skeletal muscle (MSNA) is evident in the pattern of MSNA with respect to the respiratory cycle. Eckberg et al. (10) first clearly demonstrated in humans the typical increases in MSNA occurring during exhalation and the subsequent decrease in MSNA coincident with inspiration. Subsequent research has helped define three potential respiratory related mechanisms that may act to modulate MSNA: 1) Direct interaction of presympathetic nuclei and inspiratory neurons, 2) reflex feedback from pulmonary or thoracic stretch receptors, and 3) cardiopulmonary baroreflex modulation related to intrathoracic pressure and venous return (23, 28, 29, 33). Indeed, the reflex pathways of each of these mechanisms overlap within the brain stem, along with both arterial baroreflex and peripheral and central chemoreceptor pathways, and, in turn, may modulate efferent sympathetic activity (5, 15).

However, whether these mechanisms and respiratory modulation of sympathetic activity, in general, affect the magnitude and patterning of the sympathetic response to prolonged chemoreflex engagement of progressive severity is relatively unknown. This question is most relevant during conditions in which ventilation is greatly increased, such as during exercise or chemoreflex stress or when ventilation is absent, such as during apnea.

Voluntary apnea is a powerful sympathetic stressor, and expiratory apnea is commonly used to confirm reactivity of MSNA while searching for an adequate nerve site (8). It is also present during water-sport activities and, in the extreme, in trained breath-hold divers (9). However, pathological apnea, as observed in sleep-disordered breathing, occurs in 5–10% of the population (39) and is, in turn, associated with an elevated risk of cardiovascular morbidity (3). Multiple mechanisms contribute to sympathetic activation during apnea, including hypoxia, hypercapnia, and an increased central drive-to-breathe (24). There is also the suggestion that the absence of ventilation itself may play an important role in sympathetic activation during apnea (32).

Previous investigations examining the role of apnea in the sympathoexcitatory response to peripheral chemoreflex stimulation have typically had participants perform voluntary end-expiratory breath-holds at rest and during various levels of hypoxia (18, 32). However, a confounding factor is the progressive increase in both peripheral and central chemoreflex activation induced during apnea itself and an inability to match the degree of chemoreflex activation between conditions with and without ventilation. Further, apnea during hypoxia is tolerated for only short durations, making the quantification of spontaneous, intermittent sympathetic activity difficult. Alternatively, apnea may be tolerated for much longer durations following hypoxic hyperventilation. Previous observations under such conditions suggest that differences in measured sympathetic outflow may exist in the presence or absence of ventilation, though the specific effects of ventilation (vs. differences in prevailing blood gases) were not identified (1).
Therefore, the purpose of the present study was to investigate the specific role of ventilation in suppressing sympathetic activation during a more severe and prolonged period of chemoreflex activation. To do so, we adapted a protocol previously devised by Fowler (12), whereby apneas are performed in series and separated with one or two breaths from a rebreathing circuit. As such, we tested the hypothesis that pulmonary ventilation inhibits sympathetic outflow such that, during apnea, the cessation of respiration would result in a greater sympathetic augmentation compared with rebreathing for the same magnitude of hypoxia and hypercapnia.

MATERIALS AND METHODS

Participants. Ten healthy male participants (25 ± 3 yr, 180 ± 9 cm, 81.1 ± 9.4 kg) were enrolled in the study after providing informed written consent. All participants were nonsmokers, and none had any history of cardiovascular or respiratory disease. Participants arrived at the laboratory at least 2 h postprandial and having abstained from alcohol, caffeine, or other stimulants for 12 h. Participants voided their bladder immediately prior to testing. The protocol was approved by both the Health Sciences Research Ethics Board at The University of Western Ontario in Canada and the research ethics board at The University of Split School of Medicine in Croatia and conforms to the standards set by the Declaration of Helsinki.

Data acquisition. Data were obtained from participants at both The University of Western Ontario in Canada (n = 5) and The University of Split School of Medicine in Croatia (n = 5). Croatian participants were part of a separate study examining autonomic control in trained apnea divers (3 divers and 2 controls). On the basis of this previous research, divers were determined to be different in their autonomic response to chemoreflex stress compared with controls (34). During testing, participants were supine and breathed through a face mask, which allowed normal mouth and/or nasal breathing (Hans Rudolph, Kansas City, MO). To ensure an airtight seal while maintaining participant comfort, the face mask was secured to the participant’s face using adhesive tape (Tagederm 1626W, 3M Health Sciences, Lodon, Ontario, Canada).

Ventilatory flow and volume were determined using a pneumotachograph (model 4813; Hans Rudolph) and differential pressure transducer (CD-12; Validyne, Northridge, CA), while respired gases were analyzed for PO2 and PCO2 by infrared carbon dioxide and optical oxygen detection (ML206; ADInstruments, Colorado Springs, CO). End-tidal values for PO2 (PETO2) and PCO2 (PETCO2) were determined using peak parameters software (Powerlab Software, ADInstruments).

Mean arterial pressure (MAP), systolic blood pressure (SBP), and diastolic blood pressure (DBP) were calculated on a beat-by-beat basis from the blood pressure waveform using finger photoplethysmography (Finometer; Finapres Medical Systems, Amsterdam, The Netherlands). Cardiac output (Q) was determined by the Modelflow method (38). R-R interval (RRi) and heart rate (HR) were calculated from a standard three-lead ECG. Total peripheral resistance (TPR) was calculated as the quotient of MAP/Q.

MSNA was assessed in the right fibular (peroneal) nerve by microneurography (16). A tungsten microelectrode (35 mm long, 20 μm in diameter, and tapered to a 1–5 μm uninsulated tip) was inserted transcutaneously into the fibular nerve posterior to the fibular head. A reference electrode was positioned subcutaneously 1–3 cm from the recording site. A suitable sympathetic nerve site was searched for through manual manipulation of the microelectrode until a characteristic pulse-synchronous burst pattern was observed. Confirmation that the recorded signal represented MSNA was determined by the absence of skin paresthesias and a signal that increased in response to voluntary apnea but not during arousal to a loud noise (8). MSNA activity was amplified 1,000x through a preamplifier and 100x by a variable-gain, isolated amplifier. The amplified, raw MSNA signal was band-pass filtered at a bandwidth of 700–2,000 Hz, sampled at 10,000 Hz and stored for offline analysis (Powerlab Software, ADInstruments, USA).

Protocols. Two protocols were performed in a random order and separated by 10 min of recovery. Protocol 1 was initiated with 5 min of baseline measurement; subjects were then switched to a rebreathing apparatus, at end-expiration, using a three-way valve. The rebreathing apparatus consisted of two 5-liter breathing bags connected to the three-way valve in parallel using a “Y” connector (VacuMed, Ventura, CA). Breathing bags were prefilled at rest with ~6 liters of the participants’ expired gases. After initiation of rebreathing, subjects were instructed to rebreathe as long as they could maximally tolerate or until termination criteria were met (PETO2 = 45 Torr and or PETCO2 = 60 Torr).

Similarly, protocol 2 started with a 5-min period of baseline measures. Subjects were then instructed to perform an end-expiratory apnea (to functional residual capacity) for as long as possible. At the same time, the three-way valve was turned to the rebreathing apparatus. Again, the breathing bags were prefilled at rest with the participants’ expired gases (~6 liters). At apnea break-point, participants were coached to take two controlled breaths from the rebreathing apparatus followed by a subsequent end-expiratory apnea. Lung volume was maintained the same between successive apneas by having the subjects refill the gas reservoir to the same extent each time. Apneas were repeated in this manner until subjects could no longer tolerate breath holding or termination criteria (same as above) were met. At this point, subjects were coached again to take two controlled breaths from the rebreathing apparatus to obtain final end-tidal gas values before being returned to room air.

Data analysis. Baseline cardiovascular and sympathetic data were averaged over at least 3 min immediately preceding rebreathing or apneas. MSNA bursts were identified as exhibiting pulse-synchrony, having an amplitude ≥ 2 times the previous interburst period and having characteristic rising and falling slopes. Burst occurrence was confirmed by visually inspecting the corresponding raw neurogram. MSNA was measured as burst amplitude (au), burst frequency (burst/min), and burst incidence (bursts/100 heart beats). Mean burst amplitude, frequency, and incidence responses were averaged over ~3 min of rest prior to each protocol, and over the entire duration of rebreathing and apneas.

End-tidal gas measures preceding apnea, during the periods of rebreathing between apneas, and immediately following the final apnea were fit using a polynomial function to calculate the change in PETO2 and PETCO2 during apneas themselves. To produce linear relationships between cardiovascular and neural outcomes with chemoreflex stimuli, and to avoid the inherent temporal delay associated with hemoglobin saturation measured by pulse oximetry, a transform of PETO2 to calculated hemoglobin saturation (ScO2) was used (30).

Commencing with the initiation of rebreathing or apneas, the amplitude of each successive sympathetic burst was summed to produce an incremental increase in total MSNA across time. This approach accounted for concurrent changes in both burst frequency and burst amplitude during each protocol. Incremental total MSNA was subsequently plotted as a function of the concurrent change in ScO2 with the linear slope of this relationship taken to represent sympathetic gain during each protocol (34). Importantly, analysis of sympathetic gain between protocols was matched for the same change in ScO2 (9 ± 5% and 8 ± 3% during apneas and rebreathing, respectively) and the same duration (127 ± 24 s during both protocols).

Statistical analysis. All statistical analyses were performed using Sigma Stat 3.11 (Systat Software, Chicago, IL). Differences between conditions and times for measures of sympathetic activity were assessed using a 2-way repeated-measures ANOVA. Where significant interactions occurred, a Tukey’s post hoc analysis of pair-wise comparisons was performed. Sympathetic gain was compared be-
in burst incidence during repeated apneas compared with rebreathing (Fig. 2; *P < 0.05). This further demonstrated by the larger total sympathetic gain observed during repeated apneas (2.29 ± 1.29%/desaturation) vs. rebreathing (1.44 ± 53%/desaturation; *P < 0.05) (Figs. 3 and 4).

The pattern of sympathetic activation during the repeated apnea protocol was also modulated by the brief periods of rebreathing between apneas, which resulted in brief intervals of sympathetic silence despite maintained chemoreflex stress. This withdrawal of sympathetic activity was timed to inspiration and prior to a fall in arterial pressure (Fig. 5).

The cardiovascular responses to the protocols are consistent with the neural activation patterns (Table 1). During apnea, there were larger increases in mean, systolic, and diastolic pressures, as well as an amplification of pulse pressure compared with during rebreathing. This larger pressor response during apneas was due to a greater peripheral vasoconstriction, as indicated by the larger increase in total peripheral resistance, compared with rebreathing (Table 1, *P < 0.05). Conversely, only rebreathing resulted in an increase in heart rate (Table 1, *P < 0.05) and subsequently cardiac output (Table 1, †P < 0.05).

**DISCUSSION**

In the current study, we demonstrate a restraint of the MSNA response to chemoreflex engagement due to the influence of ventilation. An augmented MSNA response during repeated periods of apnea was further associated with a larger degree of peripheral vasoconstriction and greater pressor response. Additionally, the act of breathing per se was associated with sympathetic withdrawal during periods of rebreathing between apneas, despite a maintained chemoreflex load. These results are consistent with the notion that the act of breathing per se is responsible for the restraint of sympathetic activity during rebreathing.

This restraint of sympathetic activity during rebreathing was not due to a primary respiratory avoidance response, as this has been shown previously to be triggered by a rapid respiratory stimulus (3, 7, 10). Rather, this restraint of sympathetic activity during rebreathing was due to the withdrawal of sympathetic activity following the cessation of ventilation. This withdrawal of sympathetic activity was timed to inspiration and prior to a fall in arterial pressure (Fig. 5).
data indicate that the sympathoinhibitory effects of ventilation persist during severe chemoreflex stress. Thus, these observations have important implications for the understanding of sympathetic and cardiovascular regulation during conditions where ventilation may be elevated (i.e., chemostimulation, exercise, etc.) or absent (i.e., voluntary or involuntary apnea).

**Dissociation of ventilatory and chemoreflex-mediated influences.** By adapting a protocol originally used by Fowler (12) to investigate drive-to-breath during breath holding, we have attempted to dissociate two modulators of sympathetic activity during apnea, namely, the progressive increase in chemoreflex activation (i.e., hypoxia and hypercapnia) and the removal of respiratory related feedback, including inhibitory input from lung and thoracic stretch receptors and respiratory related baroreflex mechanisms. Through peripheral and central chemoreceptors, decreased arterial oxygen and increased arterial carbon dioxide both independently cause sympathetic activation (35), whereas ventilation itself is a powerful, within-breath, modulator of sympathetic outflow (6, 10, 28, 29, 33). It has been shown previously that with low-frequency, deep breathing, there is an increased within-breath ventilatory modulation of MSNA (28). However, when sympathetic activity is analyzed under these circumstances across multiple breaths, the sympathetic activity “lost during inspiration [is] gained during expiration” as represented by augmented burst amplitudes during expiration (28). In this model, there is no net loss...
of MSNA. Similarly, Shoemaker et al. (31) demonstrated no change in basal sympathetic activity during voluntary hypoxic hyperventilation. Although these previous data suggest no broader impact of respiratory pattern on total sympathetic activity at rest, here, we demonstrate that during increased chemoreflex drive, the absence or presence of ventilation contributes significantly to the degree of sympathetic outflow.

Other attempts to distinguish the role of ventilation, independent of chemoreflex stress, on sympathetic activation have produced varied results. Khayat et al. (21) showed that the magnitude of sympathetic activity during apnea was the same in control and lung transplant recipients. Their data suggest that lung stretch receptors appear not to impact global sympathetic activation patterns during apnea, although breath holding was performed at end-expiration and a direct comparison to periods of similar chemoreflex stress accompanied by ventilation was not made. Alternatively, Somers et al. (32) demonstrated that sympathetic activation was greater during a hypoxic stress when accompanied by apnea vs. hypoxia alone. However, this analysis was performed on small segments of data (~13.5 s) and could not account for any further rise in CO₂ or decrease in O₂ during the apneic periods themselves. Irrespective of this limitation, these data are consistent with our own findings.

Mechanisms of ventilatory sympathetic restraint. The finding of heightened sympathetic drive for a given state of chemostimulation during apneas, compared with rebreathing, may not appear that surprising in light of the end-expiratory nature of the apneas themselves. In relation to the known respiratory modulation of MSNA, such apneas would be expected to relate to the end-expiratory phase of normal respiration, when MSNA is typically highest (10). With no immediate inhibitory phase during inspiration, heightened MSNA would, therefore, continue unchecked. One limitation to consider is the removal of gas from the rebreathing circuit by the gas analyzer, which may have reduced lung volume prior to each successive apnea, resulting in successive increases in MSNA. However, as can be gleaned from Fig. 3, MSNA augmentation occurred immediately with the first apnea when removal of volume from the reservoir would have had no impact. As such, removal of volume from the rebreathing circuit by the gas analyzer, or small differences in end-expiratory volumes, did not affect the pattern of results.

A second mechanism that may contribute to differing magnitudes of sympathetic activation during rebreathing and apneas may be differences in perceived stress between protocols. The increasing chemoreflex stress during both apnea (11) and rebreathing (25) is known to cause an increase in respiratory distress, as measured by a visual analog scale (VAS). However, the act of breathing itself may relieve some of this stress,
as demonstrated originally by Fowler (12) and subsequently quantified by Flume et al. (11). In the current study, rebreathing between apneas enabled subsequent breath holding despite elevated chemostimuli. Flume et al. (11) further demonstrated that during this period of rebreathing, subjects’ self-reported VAS scores were also lower. Sympathetic activity has also been shown to be increased with the degree of perceived stress (2). In this way, a higher level of perceived stress during apneas may have contributed to a higher degree of sympathetic activation. However, on the basis of the subject-by-subject responses, three of the participants were highly trained apnea divers, yet they demonstrated the same pattern of results as nontrained participants (augmented MSNA during apneas vs. rebreathing). These divers train on a repetitive basis to tolerate apnea, and thus they may have a reduced level of perceived stress during apneas (7, 19). The similarity between this small group of divers and nondivers suggests a potentially minor role of perceived stress in sympathetic augmentation during apneas. Further, the sympathetic response to stress is highly variable with a continuum between responders and nonresponders (4) with increased stress causing a reduction in sympathetic outflow in some cases (8). As we did not record levels of perceived stress, it remains unresolved if this is a contributing factor to the augmented sympathetic response during apneas compared with rebreathing.

Finally, baroreflex mechanisms may also contribute to the differing degrees of sympathetic activation between conditions. The larger cardiac output response during rebreathing may have acted upon cardiopulmonary baroreceptors to inhibit the increase in MSNA. However, it is unlikely that arterial baroreceptor activation plays a role in the difference between conditions. The significant pressor responses during both rebreathing and apneas indicate arterial baroreflex resetting during both conditions, with the larger pressor response during apneas, demonstrating a greater resetting compared with rebreathing. Such resetting has been previously shown to occur during apnea without a decrease in baroreflex sensitivity (26).

Therefore, in the current context, where there is a progressive increase in both blood pressure and MSNA, a progressive resetting must occur as well. Although chemoreflex-mediated resetting of the baroreflex has been shown (17, 35), it does not explain the greater resetting during apnea vs. rebreathing in the current study. It is possible that a mechanism related to central command acts to further reset the sympathetic baroreflex during apnea, similar to what is observed during exercise (27, 36). This concept of central command may be linked to the greater “drive-to-breathe” and respiratory distress during chemoreflex activation, which is blunted during rebreathing, but not apneas. It has yet to be determined whether such additional resetting occurs during obstructive apneas during sleep, or if the volitional aspect of wakeful breath holding is the contributing factor.

**Sympathetic withdrawal following apnea.** During periods of rebreathing at end apnea, inspiration was associated immediately with sympathetic withdrawal, similar to previous reports (1, 37). However, this neural silence occurred in the presence of high blood pressure and a maintained or elevated chemoreflex load compared with the previous period of apnea. As such, the restoration of blood gases (and removal of the chemoreflex stress) does not play a role in this regulation. Further, while the abrupt silencing of MSNA was simultaneous with inspiration, and the negative pressure generated by inspiration at end apnea could be expected to facilitate venous return, cardiac output is actually decreased transiently (13, 14). In addition, previous research has demonstrated respiratory modulation of MSNA during both normal and positive pressure ventilation (23, 33). The use of positive pressure ventilation causes a reversal of the impact of “inspiration” on venous return and atrial filling. As such, these previous data counter the suggestion that the cardiopulmonary baroreflex plays a predominant role in the silencing of MSNA at end apnea. Also, the abrupt cessation of MSNA with inspiration does not fit with the time delay required for translocation of blood through the pulmonary circulation to the arterial circulation and activation of arterial baroreceptors. Further, the work of Badra et al. (1) demonstrates little involvement of the arterial baroreflex in respiratory related modulation of MSNA. Therefore, activation of baroreflex mechanisms is likely not responsible for sympathetic withdrawal upon inspiration despite continued chemoreflex drive.

Therefore, the question remains, why is sympathetic activity silenced with inspiration following apnea despite a maintained chemoreflex stimulus, whereas augmented MSNA continues throughout the duration of rebreathing? As with the increase in sympathetic activity during apnea, baroreflex resetting may play a role in postapnea sympathetic withdrawal as well. Muetter Swift et al. (26) demonstrated that after apnea, there is an immediate downward resetting of the baroreflex accompanied by a transient (~10–30 s) decrease in baroreflex sensitivity (26). Although the mechanisms causing this immediate resetting of the sympathetic baroreflex remain unclear, it is possible that fulfillment of the drive-to-breathe, alleviation of respiratory distress, and a decreased central command may play a role.

In conclusion, the current data demonstrate that ventilation restrains sympathetic activity during progressive and severe chemoreflex stress. The nature of this restraint is not directly evident from these data; however, on the basis of the timing of neural silencing during the rebreathing period between apneas, and previous research, it is likely that central respiratory-sympathetic integration and the influence of central command play a role. The augmented sympathetic response to apnea and associated pressor response may exert additional stress on the cardiovascular system in pathological situations.

**Perspectives and Significance**

In the present study, the augmentation of MSNA during both rebreathing and apneas resulted in pronounced pressor responses. However, the larger sympathetic response during apneas was further associated with a greater increase in peripheral vasoconstriction and blood pressure well into what would be considered the hypertensive range. Although the simultaneous rise in diastolic blood pressure and sympathetic activity indicates a progressive shift of the sympathetic baroreflex during both conditions, this resetting was again greater during apnea. These enhanced cardiovascular responses during apnea may be one mechanism contributing to the increased incidence of cardiovascular pathology observed in patients with sleep apnea (3).
Further, intermittent hypoxia has been shown to cause augmented MSNA and increased blood pressure at rest (22) and in response to chemostimuli (20, 22) in otherwise healthy individuals. This approach has been used as a model to study sleep apnea. However, the data from the current study demonstrate that the cessation of breathing per se also plays an important role in the sympathetic response to apnea and as such, should be taken into consideration when interpreting results, particularly negative findings, from intermittent hypoxia protocols where the presence of ventilation may act to suppress sympathetic activation and mute subsequent cardiovascular responses. Additionally, the ventilatory sensitivity to hypoxia and hypercapnia should be taken into account when interpreting the sympathetic cardiovascular consequences to these stimuli. Finally, these data may be relevant to the study of sleep-disordered breathing and sleep apnea in that the absence of breathing, combined with oxidative stress, may compound the autonomic and cardiovascular consequences of apnea.

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